



# Characterization and antibacterial activity of endophytic fungi isolated from *Avicennia marina*

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## Abstract

In the present study, we investigated the antibacterial activity of potent endophytic fungi isolated from the roots of *Avicennia marina*, a mangrove species collected from the Gulf of Kutch, Gujarat, India (23°01'58.6"N 70°09'27.3"E). About thirteen fungal endophytes were isolated from the roots of *A. marina* utilizing Czapek dox agar medium (10% w/v, NaCl and pH, 7.2). Antibacterial activity against selected pathogenic bacterial test cultures was checked by agar well diffusion method using ethyl acetate extracts of 3 weeks old fungal cultures. Very strong antibacterial activity was recorded against a diverse range of bacterial pathogens, including *Shigella flexneri*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* and *Klebsiella pneumoniae*. Six fungal isolates, which displayed the antibacterial activities, were subjected to the molecular identification. The rRNA internal transcribed spacer (ITS) sequences with high similarity indexes were obtained from GenBank. The phylogenetic analysis of these fungal isolates was performed using the maximum likelihood method, which showed that the selected six fungal isolates belonged to *Alternaria*, *Aspergillus* and *Penicillium*. Three fungal extracts of isolate TR-1 10(7), KR-1 10(14) and TR-2 10(32) displayed broad-spectrum antibacterial activity. Among the three isolates, the crude extract of *Penicillium sp.* TR-1 10(7) showed broad-spectrum activity against all the test cultures used in the study. The findings suggest that the isolated endophytic fungi from *A. marina* could be a reservoir of unusual bioactive metabolites. This is the very first report on the antibacterial potential of endophytic fungi, derived from *Avicennia marina*- belonging to the Gulf of Kutch (Western India) to the best of our knowledge.

**Keywords** Antibacterial · *Avicennia marina* · Bioprospecting · Endophytic fungi · UPGMA

## Abbreviations

ITS	Internal transcribed spacer
UPGMA	Unweighted pair group method with arithmetic mean
PCR	Polymerase chain reaction
ML	Maximum likelihood method
MIC	Minimum inhibitory concentration

## Introduction

Mangrove vegetation covers 18 million hectare of coastline around the world. It is one of the most productive ecosystems found between tropical and subtropical areas of land and the coastal sea regions (Spalding 1997). Compared to global mangrove cover, the total mangrove cover in India is estimated to be around 2.7% (Giri et al. 2011). These forests are uniquely adapted to harsh tides, severe airstreams, high temperatures, oxygen deficiency, muddy soil, and high saline conditions (Kathiresan 2010). Surrounded by the Arabian Sea, Gujarat is located on India's west coast with the second largest tidal forest in India, following Sunderbans (Bhatt et al. 2009). It has the most extended coastline in India, with approximately 1650 km, supporting a diverse range of marine vegetation and wildlife.

Mangroves are known to possess various medicinal properties (Bandaranayake 2002). Earlier studies showed that all parts of mangroves, including the bark, stems, leaves etc., can be used for different therapeutic purposes such as antibacterial,

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ulcer-healing, anti-inflammatory properties, etc. (Suito et al. 1985; Abeysinghe 2010; Roome et al. 2008). Apart from mangrove plants, endophytic microbial flora which mainly consists of endophytic fungi, has been seeking the attention of various researchers and scientists due to their useful metabolites and therapeutic advantages, including anticancer, antibacterial and antimicrobial activities (de Souza Sebastianes et al. 2013; Elissawy et al. 2019). Fungal endophytes reside asymptotically in the host plant's living tissues without causing any harm (Aly et al. 2011). In addition, they help the host plant to survive under harsh environmental conditions by producing some metabolites including colletotric C, colletotric B, chaetochromone D, 3-hydroxy-5-methoxy-2, 4 6-trimethylbenzoic acid, and 8-hydroxy-pregaliellalactone B (Douanla-Meli et al. 2013; Chen et al. 2019) as well as by modifying their metabolic pathways. These metabolites are either released in the environments or remain associated with endophytic fungi as intracellular products, thus providing long-term sustainability to endophytic fungi and co-host plants for survival in the adverse marine ecosystem (De Souza Sebastianes et al. 2013). Earlier reports suggested that these metabolites offer several therapeutic applications for cancer treatment as well as developed as antimicrobial targets (Fox and Howlett 2008; De Souza Sebastianes et al. 2013). Similarly, Elissawy et al. (2019) reported anticancerous activity of endophytic *Aspergillus* sp. from these mangroves against Caco-2 cancer cells. When endophytes coexist with a host plant, the host develops the capability to survive in presence of harsh environmental conditions. (Amin 2016; Hartley et al. 2015; Postshangbam et al. 2017). However, a true potential of this mangrove associated endophytic fungi remains untapped despite a diverse range of therapeutic applications offered by them (Debbab et al. 2011). Recent research has sparked intense interest in novel biotechnological approaches, centred on the use of bioactive chemicals extracted from endophytes from the number of biological potentials, like antimicrobial, antioxidant, anticancer, anti-diabetic and anti-inflammatory compounds (Ukwatta et al. 2019). Due to the rising prevalence of antibiotic drug resistance, there is a persistent demand for new antimicrobials with improved pharmacological properties. In order to acquire new chemical derivatives, untapped sources with promising organisms should be investigated. Thus, the current study aimed to explore the antibacterial potential of endophytic fungi, derived from the roots of *Avicennia marina*, a tropical mangrove plant typically found in one of the untouched habitats of the Gulf of Kutch, Gujarat, India.

## Materials and methods

### Sample collection

Healthy and mature roots of *Avicennia marina* were collected from three mangrove sites: Mundra (22°46'23.0"N 69°42'14.2"E), Tuna (22°58'16.0"N 70°06'11.0"E) and Kandla (23°01'58.6"N 70°09'27.3"E) from Kutch region, Western India. Root samples were transported to the laboratory in a sterile plastic container. Small sections of 0.5 cm<sup>2</sup> were cut using a sterile blade at the time of processing.

### Isolation of fungal endophytes

The harvested root sections were sterilized using protocol of Kjer et al. (2010). After cleaning with water, the root sections were treated with ethanol (70%, v/v) for 60–120 s. Later, the root sections were immersed in sodium hypochlorite (4%, v/v) for 60 s and washed thoroughly with sterile distilled water. At last, all the treated root sections were dried using sterile Whatman filter paper. The sterilized root pieces were cultivated on Czapek dox agar medium (NaNO<sub>3</sub> 3 g, K<sub>2</sub>HPO<sub>4</sub> 1 g, MgSO<sub>4</sub> 0.5 g, KCl 1 g, FeSO<sub>4</sub> 0.01 g, sucrose 20 g, artificial sea salt 100 and 20 g agar in 1L distilled water) at 25–30 °C for 7 days.

After 7 days of incubation, the growth of fungal hyphae was recorded, and the isolates were subcultured on Czapek dox agar medium. Further, the fungal isolates were characterised with respect to their morphological characteristics including colony colour, elevation, form, margin etc.

### Fermentation and extraction

Four fungal plugs were cut from each culture plate, which were transferred into medium (100 ml) and incubated overnight. To assess the antibacterial potential of fungal endophytes, four different culture media including Czapek dox broth, Rose Bengal broth, Potato Dextrose broth, and Sabouraud's broth were inoculated and incubated at 30 °C for 21 days. The culture broth was harvested and filtered using Whatman filter paper no.1. Metabolites were extracted by solvent extraction method using twice the equal volume of ethyl acetate. The extracted crude fungal metabolites were dried and dissolved in dimethyl sulfoxide (DMSO) to check the antibacterial activity.

### Study of antimicrobial activity

Agar well diffusion method was used to screen potent antimicrobial fungal endophytes (Bauer et al. 1966). All the crude extracts were tested against different pathogenic

bacteria including *Staphylococcus aureus* (MTCC 737), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 3384), *Shigella flexneri* (MTCC 1457), and *Bacillus cereus* (MTCC 430). Each plate was subsequently inoculated with a test microorganism (100  $\mu$ l of approximately  $10^6$  CFU/ml) and incubated at 37 °C for 24 h after adding the crude fungal endophytic extracts and control. The size of the well was 8 mm in diameter and the resulting zone of inhibition was measured in mm with ruler. In this study, streptomycin sulphate and DMSO were used as a positive and negative control, respectively.

### Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA). For comparison with a significance level  $< 0.05$ , one-way analysis of variance (ANOVA) was employed, followed by Tukey's multiple comparison test. All of the experiments were carried out in triplicates.

### DNA extraction and PCR amplification

Six endophytic fungal strains (TR-1 10(1), KR-1 10(2), KR-1 10(3), TR-1 10(7), KR-1 10(14), and TR-2 10(32)), which have the most significant antibacterial activity, were identified. DNA amplification and partial sequencing of 18S rRNA using ITS 1 and ITS 4 primers were carried out. The obtained sequences were aligned with GenBank sequences using BLAST (Basic Local Alignment Search Tool). High similarity sequences with  $> 98\%$  similarity index were selected from BLAST and used for phylogenetic analysis of the isolated fungal endophytes.

### Phenotypic analysis

A biometric approach was used to cluster the isolates based on their phenotypic characters using PAST software (Hammer et al. 2001). The Unweighted Pair Group Method with Arithmetic averages (UPGMA) using the Jaccard similarity coefficient and cluster analysis was implemented to analyse the morphological characteristics (Cruz 2008).

### Phylogenetic analysis

Fungal ITS-rDNA sequences of the six fungal endophytes were deposited in GenBank. Phylogenetic analysis was performed using MEGA X (Pereira et al. 2009), where 1000 bootstrap and the maximum likelihood (ML) method was chosen as a set parameter for clade calculations.

## Results

### Identification of fungal endophytes

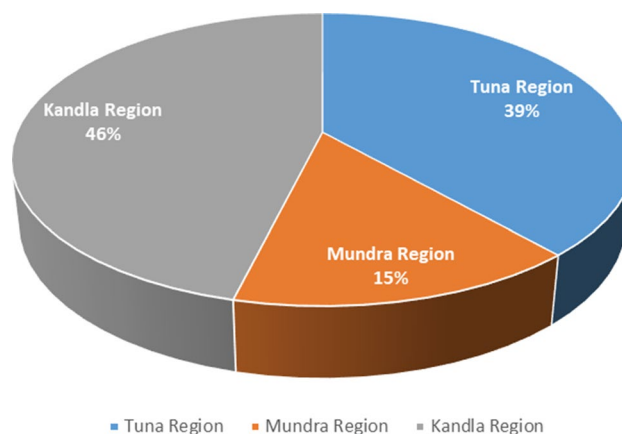
In the present investigation, thirteen endophytic fungi were isolated from the healthy roots of *A. marina*, collected from the Gulf of Kutch, Gujarat, India. Maximum number of the fungal isolates (46% of the total) was derived from Kandla region (Fig. 1). The isolates were characterized morphologically with respect to colony appearance, mycelium colour, hyphae diameter, number of nuclei and reverse media colour (Table 1). Mostly three different spore colors; green, yellowish-brown and black were observed in the morphological examination of the isolates with green being the most common (Fig. 2). Most of the isolates had powdery and velvety appearance, while a very few had woolly texture.

### Phenotypic analysis

Phenotypic analysis was carried out using the Cluster analysis and UPGMA method. A cladogram was developed based on the morphological comparison among samples and taxa. A cluster analysis revealed that taxa were divided into two primary groups (Fig. 3).

### Phylogenetic analysis

Six of the most potent endophytic fungal isolates, showing the antibacterial activity, were subjected to molecular characterization using PCR and sequencing technology. BLAST analysis was carried out to match the similarity of the obtained sequences with the known sequences at GenBank (NCBI). The above analysis revealed that, the isolates belonged to three distinct genera *Aspergillus*, *Penicillium*, and *Alternaria*.



**Fig. 1** Percentage number of isolates obtained from each sample collection sites of Gulf of Kutch, Gujarat, India

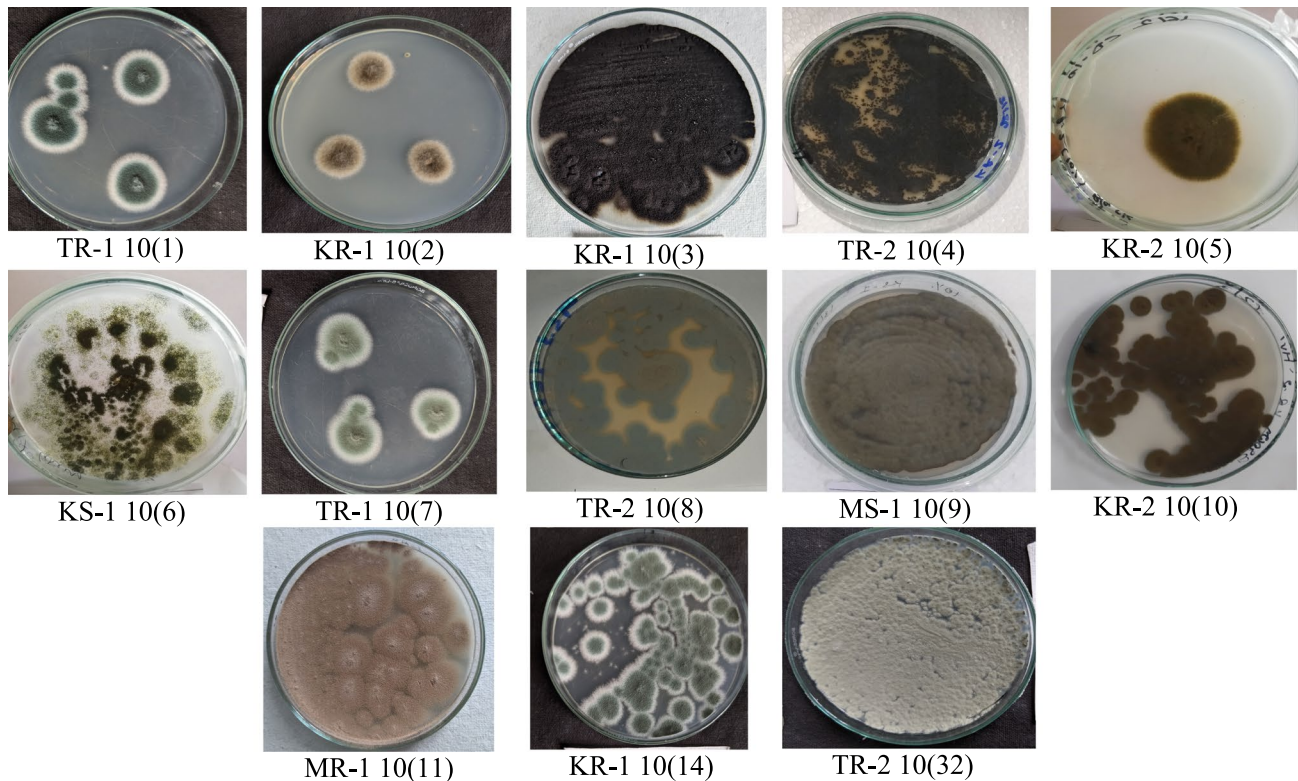
**Table 1** Colony characteristics of the fungal endophytes

Isolate ID no	Taxonomic class of Fungi	Colony appearance (Young-upon ageing)	Colony morphology	Shape	Size	Type of hyphae	Number of nuclei	Diameter of hyphae	Reverse media color
TR-1 10(1)	Ascomycetes	Green-dark green with white periphery	Dry Powdery	Circular	medium	Septate	Multinucleated	3.1 $\mu\text{m}$	Dirty White
KR-1 10(2)	Deuteromycetes	Light brown—dark brown	Woolly and Fluffy	Circular	medium	Septate	Multinucleated	4.22 $\mu\text{m}$	Black
KR-1 10(3)	Deuteromycetes	Green-black	Dry, Woolly	Circular	medium	Septate	Multinucleated	4.0 $\mu\text{m}$	Black
TR-2 10(4)	Ascomycetes	Shiny Black	Smooth, Shiny	Circular	Punctiform	Septate	Multinucleated	1.6 $\mu\text{m}$	Black
KR-2 10(5)	Deuteromycetes	Olive green-black	Smooth, Velvety	Circular	medium	Septate	Multinucleated	4.1 $\mu\text{m}$	Black
KS-1 10(6)	Ascomycetes	Yellowish Green—Dark Green	Dry Powdery	Filamentous	medium	Septate	Multinucleated	4.2 $\mu\text{m}$	Black
TR-1 10(7)	Ascomycetes	Light Green-Dark Green with white periphery	Smooth, Velvety showing folds	Circular	medium	Septate	Multinucleated	4.6 $\mu\text{m}$	Dirty White
TS-2 10(8)	Ascomycetes	Grey-buish green	Smooth, Velvety	Circular	medium	Septate	Multinucleated	4.0 $\mu\text{m}$	Dirty White
MS-1 10(9)	Deuteromycetes	Brown-black	Dry, Woolly	Circular	medium	Septate	Multinucleated	4.7 $\mu\text{m}$	Black
KR-2 10(10)	Deuteromycetes	Dark Brown-black	Smooth, Velvety	Irregular	medium	Septate	Multinucleated	4.4 $\mu\text{m}$	Black
MR-1 10(11)	Ascomycetes	Light Yellow-brown	Dry Powdery	Circular	Large	Septate	Multinucleated	2.9 $\mu\text{m}$	Yellowish
KR-1 10(14)	Ascomycetes	Green-dark green with white periphery	Dry Powdery	Circular	medium	Septate	Multinucleated	3.7 $\mu\text{m}$	Dirty White
TR-2 10(32)	Ascomycetes	Grey -light green	Smooth, Velvety showing folds	Circular	Small	Septate	Multinucleated	4.3 $\mu\text{m}$	Dirty White

For phylogenetic tree construction of *Aspergillus sp.*, two sequences from the isolated fungi and 21 different reference sequences derived from NCBI Genbank were used. The results revealed that ML tree contained 23 sequences with total 689 positions present. TR-1 10(1) and KR-1 10(14) belonged to two different clades but were closely related. TR-1 10(1) was closely related to *Aspergillus versicolor* F38B (MT102258), and KR-1 10(14) was associated with *Aspergillus sp.* Y38-1 (KP872505) with 100% bootstrap support (Fig. 4).

*Alternaria sp.* isolates were classified into two main clades where, 16 different reference sequences were derived from NCBI, and two sequences of isolated fungi were selected to construct a phylogenetic tree. KR-1 10(2) sequence was associated with *Alternaria alternata* (MT489691) and KR-1 10(3) sequence was associated with *Alternaria sp.* (MT982648). About 611 positions are included in this data set (Fig. 5).

About 24 reference sequences and 02 sequences of the isolated fungi were selected to construct the phylogenetic



**Fig.2** Endophytic fungal isolates obtained from Tuna region, Mundra region and Kandla region of Kutch, Gujarat, India

tree of *Penicillium sp.* About 497 positions were included in the data set. While TR-1 10(7) was closely associated with *Penicillium oxalicum* (HM452308) with 99% bootstrap support, TR-2 10(32) showed the same level of similarity with *Penicillium sp.* (MT649559) (Fig. 6). The analysis further revealed that all the isolated fungal endophytes belonged to phylum *Ascomycota*. Four isolates, TR-1 10(1), TR-1 10(7), KR-1 10(14), and TR-2 10(32) represented order *Eurotiales*. While TR-2 10(32) and KR-1 10(2) had the highest bootstrap support of 100%, KR-1 10(2) and KR-1 10(3) represented order *Pleosporales*. The ITS sequences data of the isolated fungal endophytes were deposited in the NCBI GenBank (Table 2).

### Antibacterial activity of endophytic fungal extracts

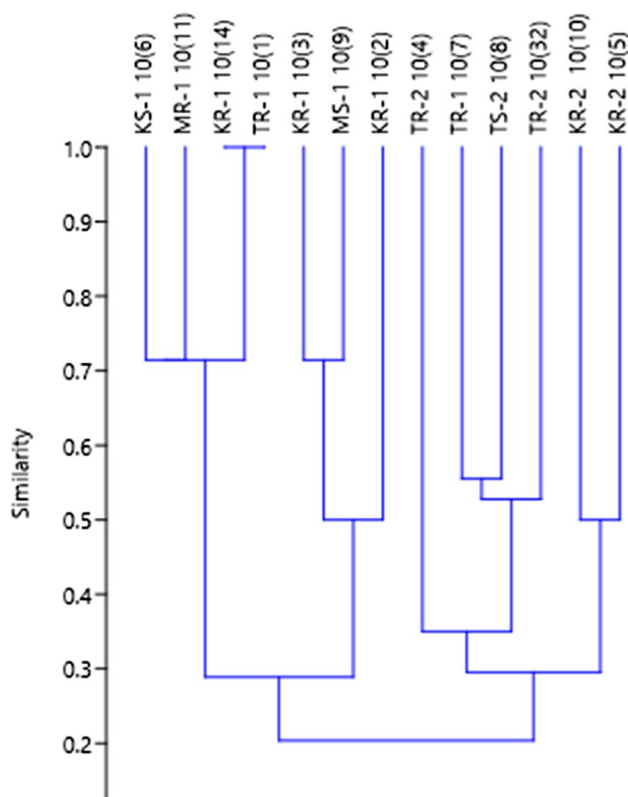
Antibacterial activity of crude metabolite extract from the endophytic fungal isolates was studied against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. cereus*, *S. flexneri* and *K. pneumoniae*. Based on the screening results, six endophytic fungal extracts were selected with impressive antibacterial activity against the pathogens. While four endophytic fungal extracts inhibited the growth of *E. coli* and *B. cereus*, three extracts suppressed the growth of *S. flexneri* and *K. pneumoniae*. Detailed results revealed that the organic solvent extracts of TR-1 10(7) and TR-2 10(32) have potent

antibacterial activity as compared to other isolates. However, KR-1 10(14) showed the average zone of inhibition against *S. aureus* (zone diameter, 22.66 mm), while extract from KR-2 10(2) was least effective in comparison to all the fungal extracts (Fig. 7). A negative and positive control was used to distinguish the results and all the experiments were conducted in triplicates. A Tukey's multiple comparison test of significance ( $p < 0.05$ ) was used to compare the mean of values (Table 3).

An independent t test of significance ( $p < 0.05$ ) was used to compare the means of the values.

### Discussion

Plants harbour a wide variety of endophytes. However, the substantial interaction between host plants and endophytes is concealed, and their functions towards their host plants are intriguing. However, the endophytic community promotes plant growth and development of plants, while also contributing to improvement of environmental conditions. Endophytes, for example, help their host plants to flourish by providing phytoremediation, biodegradation, growth hormones, and nutrient cycling, which in turn reduces the amount of debris in our environment (Nair and Padmavathy 2014).



**Fig. 3** UPGMA- dendrogram through cluster analysis using Jaccard similarity coefficient based on morphological characteristics of all the isolates obtained from roots of *A. marina*

Almost all the parts of plant harbour a variety of endophytic fungi. However, it is worth noting that the type of fungal species and the quantity of the fungal isolates vary greatly amongst plants (TJ et al. 2019). In our study, fungal endophytes were classified on the basis of their morphological characters like shape, size, texture, colour, type of hyphae and number of nuclei. To determine the significance of the morphological features on species analysis, multivariate analyses were performed. The Jaccard similarity coefficient and unweighted paired group (UPGMA) method were used to develop a cladogram. Colour of the fungal mycelia and texture of the colony were significant for cladogram construction. Two chief clusters were constructed based on the variations in the morphological features of the isolates (Fig. 3).

Six fungal isolates belonged to phylum *Ascomycota* as revealed by ITS sequences. Earlier reports also confirmed wide association of phylum *Ascomycota* as the endophytic partner with plants (Crozier et al. 2006; He et al. 2012; Koukol et al. 2012). However, a very small proportion of these fungal communities are amenable to cultivate in vitro using traditional microbiological protocols (Amann et al. 1995). Nature provides all kinds of nutrient supplements to the microbial flora and also helps them to adapt to harsh

conditions. In contrast, all kinds of microbes do not grow under laboratory conditions. The possible reason for such type of microbial behaviour lies in the lack of knowledge for culture conditions which include nutrient deficiencies, microbial handling and storage, etc.

Since past three decades, drug resistance problems have been increased, which made the pathogens more virulent for society (Cui et al. 2015). Thus, there is a constant need for a novel approach to gain more powerful pharmaceuticals. Handayani et al. 2017 has reported significant antibacterial activity by mangrove endophytic fungi isolated from Bungus, West Sumatra. Also, an endophytic fungus from Sundarban mangrove forest plant has also displayed extraordinary antimicrobial property against selected pathogens (Nurunnabi et al. 2020). Recently, Okla et al. (2021) reported inhibition in the growth of *S.aureus*, and *E. coli* by the root extracts of *A. marina*.

Our own findings confirmed that diversified endophytic fungi of mangrove species could also be used for therapeutic applications. Similarly, a Dichlorodiaportin, produced by an endophytic *Aspergillus sp.*, displayed significant antibacterial activity against *S.aureus* and *B.subtilis* (Chen et al. 2018). Further, several other endophytic genera have been reported to possess similar type of growth inhibitory activity against pathogens like *Botryosphaeria*, *Guignardia*, *Fusarium*, *Eutypella*, *Phomopsis* and *Penicillium* (Phongpaichit et al. 2006; Bernardi-Wenzel et al. 2010; Rhoden et al. 2012). According to Li et al. (2005), a set of Chinese researchers obtained 130 fungal endophytes from different medicinal plants having potent anticancer and antifungal properties. They found that about 30% of fungal endophytes possess antifungal properties, while 9.2% of the endophytic extract displayed antitumor activity. This study indicates the existence of fungal compounds linked to the host plants as earlier reports confirmed that the endophytic fungi are the potent producers of essential metabolites, which is also linked to the hosts (Kusari et al. 2013). Recent study revealed that *Penicillium aculeatum* effectively inhibited the growth of *Salmonella sp.* and *B. subtilis*. In addition, endophytic *Penicillium* had also been considered as a hub of essential biological metabolites like antimicrobials (Huang et al. 2017). Penochalasin K obtained from *Penicillium chrysogenum*, showed potent activity against *R. solani*, *C. gloeosporioides*, *C. musae*, and *P. italic* (Zhu et al. 2017). Similarly, Penicoffeazine A isolated from endophytic *Penicillium coffeae*, which resides on the leaves of *Laguncularia racemosa*, have shown growth inhibition of *F. oxysporum* and *Colletotrichum gloeosporioides* (Cao et al. 2019). Also, Zang et al. 2014, reported a new depsidone metabolite Phomopsidone A from mangrove endophytic fungi, whereas, a novel antimicrobial sesquiterpene have recently been isolated from Chinese mangrove plant (Quin et al. 2020).

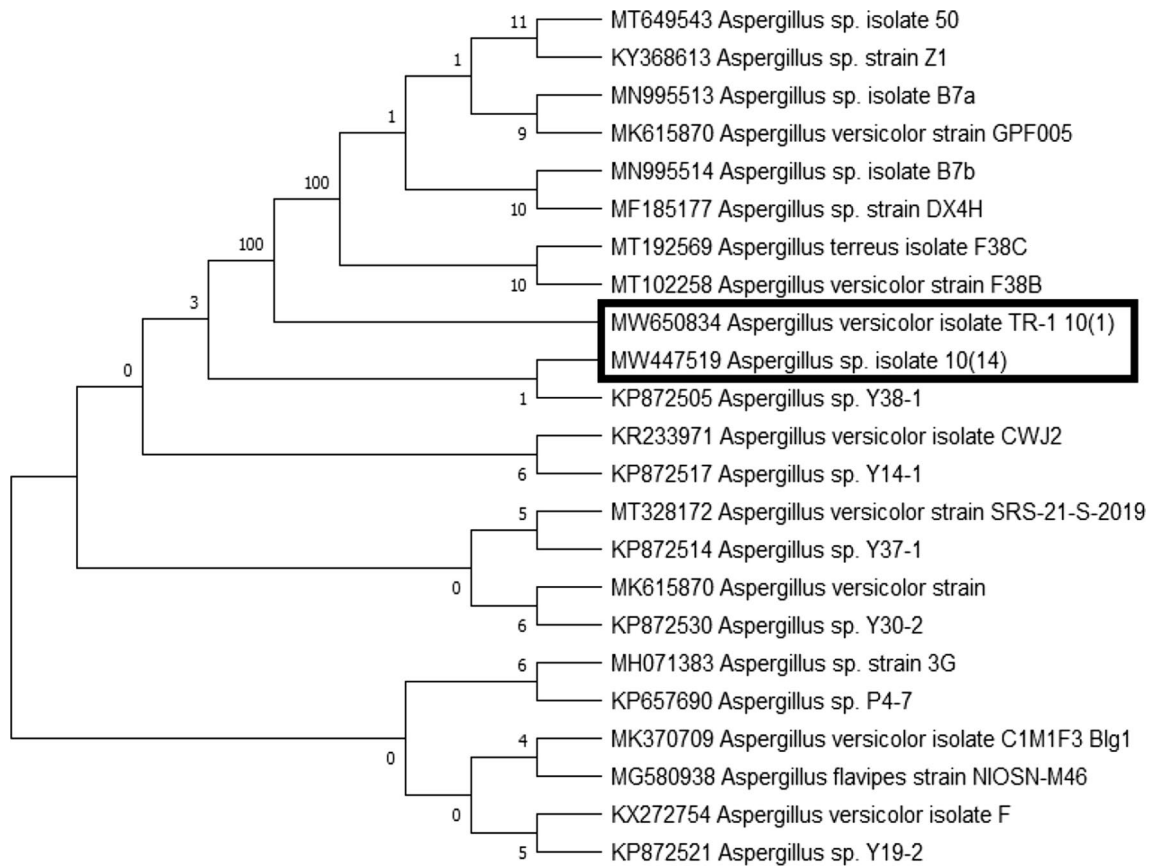


Fig.4 Phylogenetic Tree of *Aspergillus* sp. by Maximum likelihood (ML) method

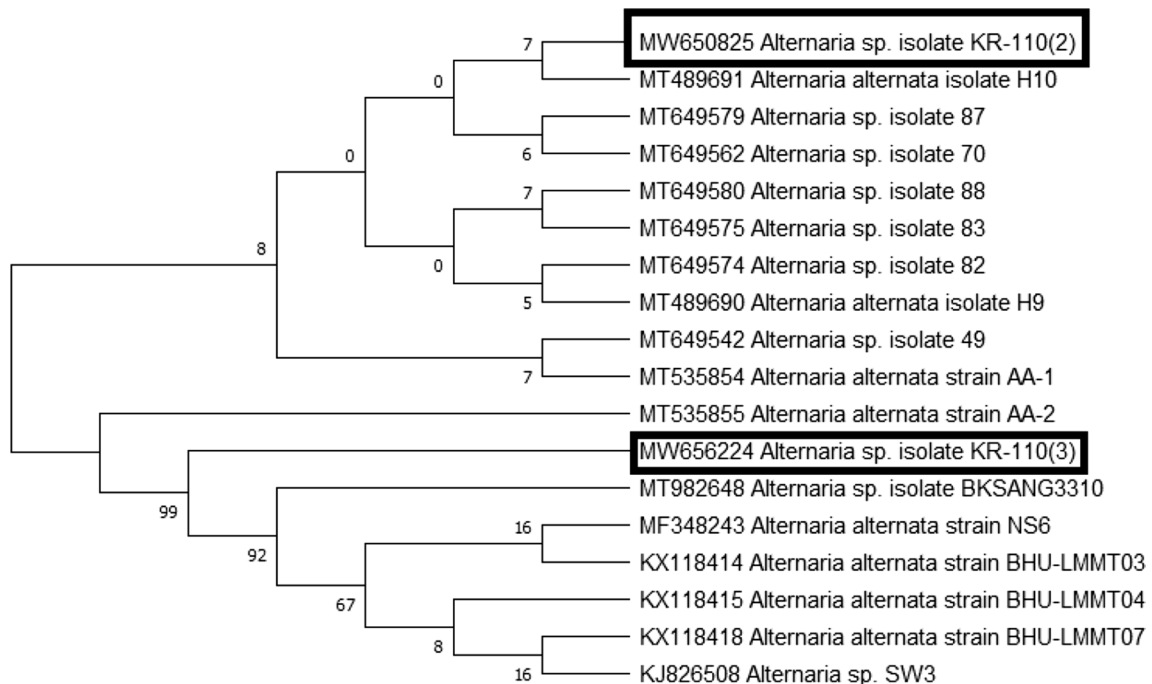
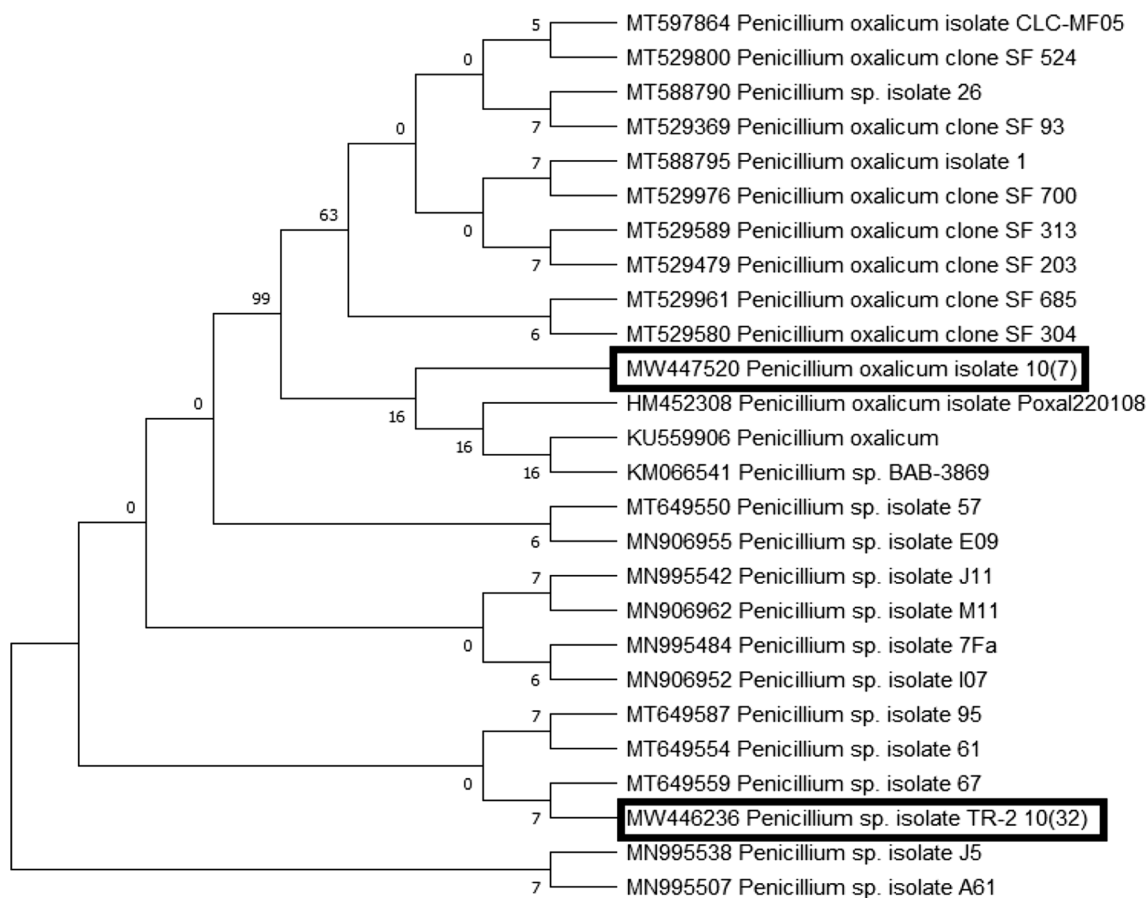


Fig.5 Phylogenetic Tree of *Alternaria* sp. by Maximum likelihood (ML) method



**Fig.6** Phylogenetic Tree of *Penicillium* sp. by Maximum likelihood (ML) method

**Table 2** Maximum nucleotide identity match for 6 fungal isolates based on ITS sequences using BLAST analysis

Sr. No	Isolate ID no	Closest identified relative	% identity	Genebank Accession no	E-value	Phylum, Class, Family
1	TR-1 10(1)	<i>Aspergillus versicolor</i>	99.27	MW650834	0.0	<i>Ascomycota, Eurotiales, Aspergillaceae</i>
2	KR-1 10(2)	<i>Alternaria</i> sp.	99.84	MW650825	0.0	<i>Dikarya, leotiomyeta, Pleosporaceae</i>
3	KR-1 10(3)	<i>Alternaria</i> sp.	100	MW656224	0.0	<i>saccharomyceta, leotiomyeta, Pleosporaceae</i>
4	TR-1 10(7)	<i>Penicillium oxalicum</i>	99.79	MW447520	0.0	<i>leotiomyeta, Aspergillaceae, Penicillium</i>
5	KR-1 10(14)	<i>Aspergillus</i> sp.	99.84	MW447519	0.0	<i>Ascomycota, Eurotiales, Aspergillaceae</i>
6	TR-2 10(32)	<i>Penicillium</i> sp.	100	MW446236	0.0	<i>Dikarya, Ascomycota, leotiomyeta</i>

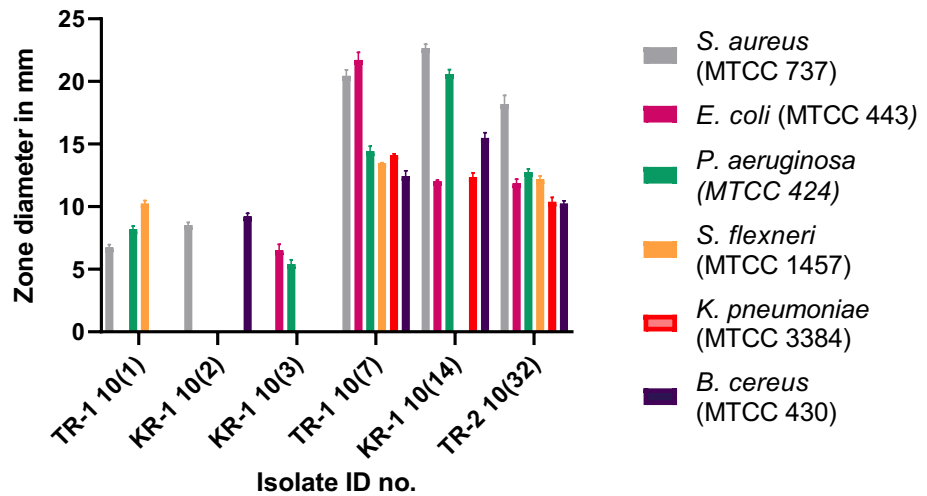
## Conclusion

The studies reported here revealed that the fungal endophytes of *A. marina*, from rarely explored habitats of the Gulf of Kutch (Western India), can emerge as the vital sources of novel therapeutic compounds. Phylogenetic analysis showed that isolated six fungal endophytes belong to diverse fungal genera including *Aspergillus* sp., *Alternaria* sp. and *Penicillium* sp. All the isolates displayed

significant antibacterial activity against a range of sensitive bacterial cultures. Such endophytes can serve as a promising source of novel antimicrobial metabolites to combat the ever increasing problem of antimicrobial drug resistance. Further, our findings can also lead to open new opportunities for researchers to investigate further about the antimicrobial mechanism and characteristics of the bioactive metabolites from endophytic fungi.



**Fig.7** Antibacterial activity of potent isolates against a range of gram positive and gram negative bacteria with agar well diffusion method



**Table 3** Antibacterial activity of the isolates

Isolate ID No	<i>S.aureus</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>S. flexneri</i>	<i>K. pneumoniae</i>	<i>B.megaterium</i>
Isolate ID No	Zone of inhibition (diameter, mm)					
TR-1 10(1)	6.74	–	8.17	10.23	–	–
KR-1 10(2)	8.53	–	–	–	–	9.21
KR-1 10(3)	–	6.5	5.4	–	–	–
TR-1 10(7)	20.45	21.71	14.42	13.45	14.11	12.44
KR-1 10(14)	22.66	12.03	20.58	–	12.36	15.48
TR-2 10(32)	18.2	11.86	12.76	12.16	10.39	10.24

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s42535-023-00582-9>.

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**Author contributions** TJ: designed and supervised the study, TN: performed the experiments, analysed the data and prepared manuscript. Both the authors have read and approved the final manuscript.

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**Data availability** The dataset generated in this study has been deposited in the NCBI GenBank under the accession numbers: MW447519, MW447520, MW656224, MW650825, MW650834 and MW446236.

**Code availability** Not applicable.

**Declarations**

**Conflict of interest** The authors declare no competing interest.

**Ethics approval** Not applicable.

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